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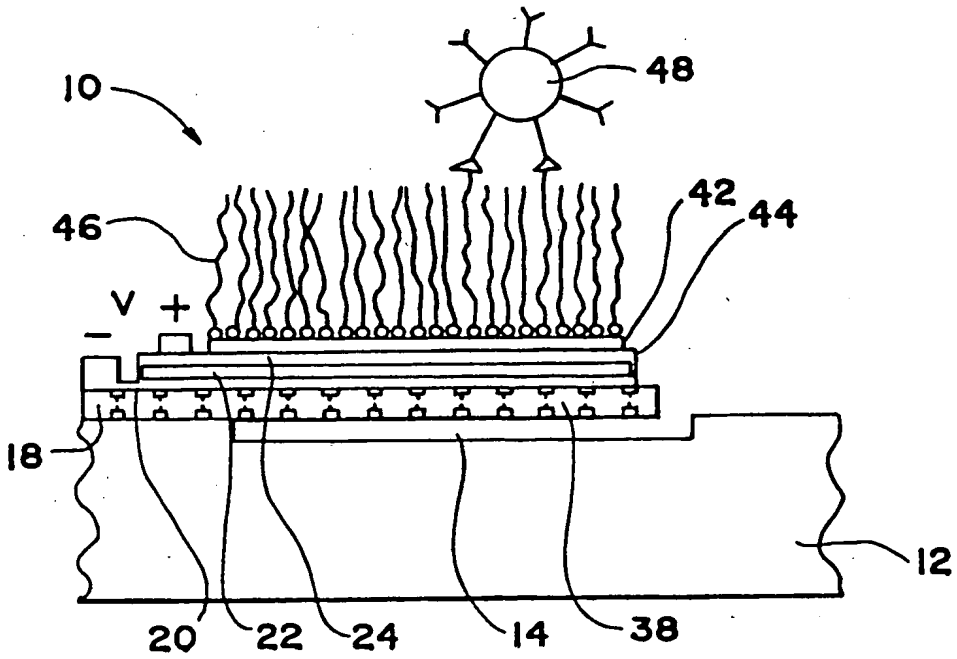
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(54) Title: MICROCANTILEVER BIOSENSOR



(57) Abstract

A biosensor includes a cantilever microbeam which responds to a chemical stimulus, binding event or mass loading with an electrical output. The microbeam is formed using a series of microfabrication processes, in the micrometer to millimeter size range and using thin deposition of piezoelectric materials.

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MICROCANTILEVER BIOSENSOR

Related Applications and Patents

The present application is a continuation in part application of U.S. Patent Application 08/852,758, filed May 8, 1997 and now abandoned and which claimed the benefit of Provisional Application No. 60/017,431 filed May 8, 1996, entitled MICROCANTILEVER BIOSENSOR. The present application is related to United States Patent No. 5,209,119, issued May 11, 1993, United States Patent No. 5,536,963, issued, July 16, 1996, which are incorporated herein by reference.

Technical Field

The present invention relates to the field of biosensors. More particularly, the present invention is a biosensor formed using MEMS technology.

Background of the Invention

The identification of disease causing mutations and the genetic characterization of infectious agents has resulted in the ability to diagnose genetic (including congenital and acquired and infectious) diseases at the molecular level. Molecular diagnostic methods, however, remain time and labor intensive, thus limiting the use and availability of routine molecular testing for patient care. "DNA testing on a chip" (i.e., the use of microchip technology for molecular diagnostics) has recently been touted as the solution to the high cost of molecular-based testing.

Advances in molecular genetics, derived principally from the human genome project, promises to revolutionize health care in the 21st century. The recognition that most human disease is a consequence of variations in the structure of DNA, whether through deleterious mutations or due to a simple difference in the sequence of DNA that predispose to disease. These observations point to the fact that molecular genetic testing is a likely final common pathway to all medical diagnoses. For this reason, one focus in health care, and for biomedical research in

the near and long term future will be to develop more advanced systems for molecular genetic based diagnostic testing.

Presently, molecular diagnostic laboratories are in their early stages of evolution. Typically these facilities exist only in large hospitals or academic medical centers, where the service offered focuses on providing a handful of tests for selected disease states. With the advent of the polymerase chain reaction, PCR, a method to amplify a precise fragment of DNA to quantities which can be easily evaluated, there has been a major improvement in the feasibility of performing routine molecular genetic testing. Consequently, PCR has become the primary biochemical technique used in molecular genetic laboratories. Although PCR, and other amplification techniques are highly specific and sensitive, they are still highly labor intensive and consequently very costly.

Thus, despite the importance of molecular genetic testing to improve patient care, the growth of this discipline is being challenged by the mandate of the health care market to fundamentally reduce the cost of genetic testing. So great are the expectations to contain costs, that given the present state of technology and automation in these laboratories, the promises of molecular genetics for patient care will not be realized because these forms of "esoteric" testing will be unaffordable.

Several factors contribute to the high cost clinical molecular genetic testing. Although microchip technology is being developed for several specific applications in the molecular genetic research laboratory, the utility of this technology for the purpose of testing has not been realized. Introduction of this technology into the clinical laboratory will dramatically decrease the cost and labor associated with molecular diagnostics, thereby increasing the availability and potential clinical applications for genetic testing. How molecular genetic testing is presently being performed, and the opportunities to improve it based on microelectromechanical (MEMS) technology is being evaluated by several research groups throughout the world.

However, based on the current methods of performing

molecular genetic testing and the costs associated with each operational step, the most significant costs are the so-called front-end which include specimen procurement and nucleic acid extraction. Specifically, collection of blood from the patient is an invasive procedure which requires a trained medical technician. Large specimen sizes are convenient for manual processing, but necessitate a large scale nucleic acid extractions which use costly reagents. Although first generation automated DNA extractors have been available, these instruments use large quantities of toxic chemicals and are not applicable to small specimens. Similarly, the test set-up, namely the assembly of the chemical reactions involved in the DNA amplification procedure, are typically done manually. Only recently have first generation robotics systems been commercialized, which are predicted to reduce the cost of labor and may also eliminate errors and increase throughput.

Of equal importance to the labor costs, however, are the costs of the reagent used in DNA amplification based chemistry. In this regard, reduction to a microscale reaction volume, such as that conceived with a microfabricated version of a thermal controlled DNA amplifier would have overall a significant impact on the reduction of the cost of genetic testing.

Exploitation of silicon as a substrate for micromachined devices is well established in the engineering fields. Microelectromechanical systems (MEMS) refers to the output of microfabricated devices including those for uses ranging from automotive parts to the airline industry. MEMS have a particular usefulness in biological applications due to their requirement for small sample sizes, low energy, and nominal forces. The increased efficiency of MEMS-based instruments, however, has yet to be realized commercially in biomedical applications, where the need for economy in manufacture, ease of operation, reduction of consumables and the mobilization of the laboratory operation to point-of-care testing are evident. While the future looks promising for the continued development of MEMS for biomedical applications, especially for the

clinical chemistry, relatively little research has been applied to the field of molecular genetics utilizing MEMS technology. There is, then, a need in the industry for a MEMS-based biosensor for use in the testing and identification of selected biological materials.

Summary of the Invention

The present invention substantially meets the aforementioned needs of the industry. The biosensor of the present invention includes two piezoelectric cantilever microbeam structures. The first structure consists of a polygonal cantilever microbeam fabricated from a suitable structural support material such as silicon, silicon nitride, aluminum, or polycrystalline silicon. This structure forms a platform onto which a piezoelectric capacitor is fabricated. The piezoelectric capacitor is comprised of an upper an lower electrode surrounding a piezoelectric or ferroelectric thin film. The top electrode is covered with an encapsulation layer to electrically isolate the active electrode surfaces of the piezoelectric capacitor.

The present invention is a cantilever microbeam which responds to a chemical stimulus, binding event or mass loading with an electrical output.

Brief Description of the Figures

Figs. 1a and 1b through 9a and 9b are fabrication steps of the microcantilever biosensor of the present invention, with the figures designated a taken along the section line in the corresponding b figure in each case, as the line 1a - 1a in figure 1b.

Fig. 10 is the biosensor of Fig. 1 including metallic gold or additional layers of polymers such as polydiacetylene intercalate the receptors for binding the specific ligand.

Figs 11a and 11b are top and side elevational views of an alternative preferred embodiment of the microcantilever biosensor.

Fig. 12a is a biosensor in a relaxed state.

Fig. 12b is a biosensor in a stressed bound state.

Detailed Description of the Drawings

The present invention relates to a method and device for the detection of inorganic and inorganic chemical reactions, (including biochemical and the interaction between microparticulate and their cognate receptors), based on the detection of spontaneous charge produced in a piezoelectric ceramic (either thin film of bulk crystal) formed in a suitable geometry to take advantage of either or both mass loading or chemically-induced mechanical stress transduction. This device consists of a miniature cantilever suspended in a manner such as a microbeam diving board fabricated with a piezoelectric stress-sensitive crystal, laminated between several materials or on its surface, coupled with an appropriate molecular recognition surface constructed of a thin film containing multiple chemical reaction sites onto which an exposed chemical, biochemical, antigen, or particulate binds or sticks. The binding or sticking effect produces a change in mass or induces a stress in the composite materials comprising the cantilever. A desirable attribute of this device is its specificity to only respond to the single external variable in which it has been designed. Upon binding, the increased mass of the cantilever may cause a change in the mechanical or electrical properties of the free-standing beam. One example is an increase in mass which alters the mechanical resonant frequency of the beam. Another effect is the change in the beam's vibrational amplitude when subjected to a loading force, whether through the specific intermolecular binding or in the context of adding a known force. The second example is a deflection of the beam due to a mechanical stress, resulting from interactions on the surface of the beam and transduced throughout the beam, to affect an overall physical deformation. Consequently, a charge is produced from this stress-induced deflection, which in turn produces a quantifiable electrical signal, confirming the chemical or physical binding event.

The microcantilever biosensor of the present invention is shown

generally at 10 in the figures. Referring to Figures 1A and 1B through 9A and 9B, a fabrication sequence of the microcantilever biosensor 10 is depicted. In Figures 1A and 1B, a trench 14 is etched in a silicon substrate 12. As depicted in Figure 1B, the trench 14 is preferably rectangular in shape and etched in the upper surface of the silicon substrate 12.

In Figures 2A and 2B, a layer of doped poly-silicon 16 is deposited in the trench 14 and then planerized to be flush with the upper surface of the silicon substrate 12. In Figures 3A and 3B, a layer of low stress LPCBD nitride 18 is deposited on the upper surface of the silicon substrate 12. In Figure 3B, the LPCBD nitride 18 is depicted overlying the trench 14, depicted in phantom.

In Figures 4A and 4B, a bottom electrode 20, a PZT layer 22, and a top electrode 24 are deposited and patterned on the upper surface of the LPCBD nitride layer 18. In Figures 5A and 5B, a layer of PECBD nitride is deposited over the full upper surface of the sensor 10. The PECBD nitride is patterned at 28 to provide electrode access to the bottom electrode 20 and the top electrode 24. The PECBD nitride layer is utilized to prevent shorting between the bottom electrode 20 and the top electrode 24.

Figures 6A and 6B depict the deposition and patterning of silver/chromium bonding pads for the bottom electrode 20 and the top electrode 24, the bottom bonding pad 30 is allowed to fill the pattern 28 to provide an electrical path to the bottom electrode 20. The top bonding pad 32 fills the pattern 28 to provide an electrical path to the top electrode 24. In Figures 7A and 7B, a PECBD nitride encapsulation layer 34 is deposited over the entire upper surface of the sensor 10. After deposition of the PECBD nitride encapsulation layer 34, a three sided parameter trench 36 is patterned in the PECBD nitride encapsulation layer 34. The trench 36 extends downward to intersect the doped poly-silicon 16 deposited in the trench 14.

In Figures 8A and 8B, a deep well etch is performed on the sensor 10. This etching is performed on the doped poly-silicon 16 that is exposed by the perimeter trench 36. The etching extends to the entire layer of

doped poly-silicon 16 that is formed in the trench 14. After etching, a cantilever beam 38 is left extended over the exposed trench 14. The cantilever beam 38 is supported at support point 40 and is unsupported on the opposed end in the two opposed sides of the cantilever beam 38 as defined by the parameter trench 36.

The final step in the formation of the microcantilever biosensor 10 is depicted in Figures 9A and 9B. The final step is the removal of the PECBD nitride encapsulation layer 34. Such removal exposes the bottom bonding pad 30 and the top bonding pad 32 for electrical connection to the cantilever beam 38.

Figure 10 is a diagrammatic representation of the cantilever beam 38 formed as depicted in Figures 1-9. In addition to the cantilever beam 38 as previously described, various surfaces which serve to immobilize the molecular recognition surface 42 are depicted. Metallic gold 44 or, alternatively, additional layers of polymers such as polydiacetylene intercalate the receptors 46 for binding the specific ligand 48.

The sensor 10 is that of a silicon based microcantilever beam 38, freely suspended above the underlying substrate 12 through a fulcrum or root 40 which is electrically isolated. The beam structure 38 itself preferably has varying dimensions but ranging from 50 to 1500 μm in length, and widths ranging from 50 to 350 μm. Overlying the silicon nitride beam 38 are a series of laminations comprised of a bottom electrode 20, an electrical insulating layer, a thin film of ferroelectric material such as lead zirconite titanate (PZT) 22, a second insulating layer, a top electrode 24, and ultimately a biomolecular recognition surface 42. Aspects of this described sensor 10 include processing of the microbeam structure through a process which includes encapsulation with polysilicon glass (PSG) 34 followed by release of a beam structure 38 by wet chemical etching resulting in a free standing beam structure 38 overlying an electrically insulated silicon substrate 12.

An alternative embodiment of the microcantilever biosensor 10 is depicted in Figures 11A and 11B. The structure is comprised of two

electrodes, top electrode 50 and bottom electrode 52. Either electrode 50 or 52 is capable of harboring the molecular recognition surface 42. In the depiction of Figures 11A and 11B, the molecular recognition surface 42 is deposited on the top electrode 50. The arrow A of Figure 11B indicates the movement of the deflected beam 54. This deflection is the result of either mass loading such as from a ligand 48, depicted in Figure 10, or stress induced transduction of the beam 54 through the immobilized recognition surface 42. Referring to Figures 12A and 12B, the effective change in confirmation of the polymer polydiacetylene, intercalated with a biomolecular receptor molecule is depicted. The change in the shape of the recognition surface 42 induces a stress into the underlying microbeam structure 38 causing the microbeam structure 38 to flex. In turn, the flexure of the beam 38 transduces force to the PZT layer 22 of the beam 38 thereby creating an electrical charge.

A portion (typically the surface of the top electrode 24) of the cantilever 38 is further coated with a molecular recognition coating such as metallic gold 44, or, alternatively, polymeric materials, organic layer, or fractionated biomolecular materials, onto which the desired analyte will bind. This molecular recognition surface 42 defines the region where the chemical reaction or the biochemical or microparticulate sensing occurs. Upon exposure to the prescribed chemical, biochemical, cellular antigen, or particulate 48 to be sensed as a function of the incremental change in mass loading on the cantilever beam 38, changes which alter its resonant frequency and/or amplitude of vibration and/or mechanically deflects the beam 38 when subjected to a input mechanical or electrical stimulus. The physical principle upon which the sensor 10 operates relies on the change in the dielectric constant of the piezoelectric material 22 consequent to the mass loading at the end of or on the surface of the cantilever microbeam 38.

A second related structure is based on the incorporation of an additional thin film of a chimeric organic material 42 such as polymer, polydiacetylene, which contains a specific biomolecular receptor 46

intercalated into the lattice structure of the polymer 42. This molecular recognition material 42 undergoes a conformational change (shape change through relative shrinking or expansion) eliciting a mechanical strain specifically when the cognate molecule (ligand 48) binds to the embedded biomolecular receptor (Figure 12b). Because this strain-sensitive thin film 42 is attached to the composite microbeam structure 38 described in the previous paragraph, the binding induced strain is transduced to the piezoelectric 22 thin film coated microbeam structure 38 which spontaneously generates a charge due to the deformation of beam 38. This detection mode therefore shows momentary piezoelectric charge induction (or voltage) response due to a specific chemical reaction.

A feature of the above invention is the use of integrated circuit processing methods to realize small biochemical detectors which can be manufactured in large batch quantities with the feasibility on on-chip electronics for most efficient electrical detection. The molecular recognition microcantilever device 10 therefore incorporates materials common to the production of both integrated circuits and piezoelectric sensors. The molecular recognition microcantilever 10 furthermore is realized using standard manufacturing methods based on thin film deposition, photolithography, chemical etching, and packaging.

Because of the overall process compatibility between the piezoelectric microsensor 10 and integrated circuits, negligible signal losses result. Furthermore, the use of on-chip multiplex sensing capabilities is possible. One way of achieving this is to form two piezoelectric microsensor structures 50, 52 with one beam 50 free to move due to a space immediately below the microbeam 50 and the other beam 52 rigidly attached to the substrate 12, as depicted in Figures 11a and 11b.

The described invention embodies a microcantilever structure which is design in both a two electrode as a well as a four electrode device. The two electrode system involves a single layer of ferroelectric material electrically connected to leads and bonding pads. In this configuration, the ferroelectric material serves both as means of physical actuating the beam,

as well as a source of force sensation. A second configuration involves a series of two ferroelectric elements, the first a driving element which is connected to a series of two electrodes and bonding pads. The second element, a sensing element is derived from a similar ferroelectric material connected to a distinct and separate set of two electrodes and bonding pads. A third configuration of the invention includes a series of three or more microbeam structures which are each electrically isolated from the other, but share a common electrical ground.

The embodiment of the invention involves the creation of a unique biomolecular recognition surface. In its simplest description, the cantilever beam structure responds to a force loading event such as a chemical binding reaction, through the deformation of the beam structure from its unloaded state, and resulting in an output of electrical charge. This phenomenon is accomplished through the effects of static mass loading of the microbeam structure consequent to the force event. This results in mechanical deformation of the beam, and in combination with gravitational force, distortion of the thin film ferroelectric material from its poled but electrically neutral state, and the consequent production of electric charge.

A second configuration of biomolecular sensing is accomplished through the same structure, however, in this setting the beam is actuated at its mechanical resonance or at its electrical resonance. For each microbeam structure, actuation of the structure to its resonance is achieved by the input of an applied voltage. This results in the oscillation of the beam at a frequency dependent upon the cycle frequency of the voltage source (function generator). At the frequency at or near the mechanical or electrical resonance, the amplitude of the output voltage as measured through the output electrode is markedly increased as compared to the voltage at nonresonant frequencies. It is at this frequency (frequency range) that the beam structure can be deemed to be most efficient in its capacity to sense masses bound to it's surface. Upon binding of a molecular species, the overall mass of the beam structure is increased,

resulting in a change in the intrinsic resonance frequency of oscillation. Altering the input frequency of the applied voltage over a prescribed range greater than or less than the resonant frequency of the unloaded beam, the characteristic increase in output voltage at the new resonant frequency is proportional to the mass loaded to the microbeam structure.

In a practical sense, these biomolecular microcantilever sensors can operate in both a fluid as well as a gaseous environment. In the case of sensing a mass loading in a fluid environment, the cantilever sensor is submerged in a prescribed volume of fluid confined by a fluid cell which surrounds the sensing element. A reference sensor, which lacks the biomolecular recognition surface, is subjected to the same fluid environment, defines the baseline or index resonant frequency of the beam structure(s) in the fluid submerged state. A second sensor (test sensor) containing the specific biomolecular recognition surface is oscillated in a same fluid environment, is permitted to react with the cognate analyte, through a mass loading event. The consequent resonant frequency of the test is measured. The measurement of quantity of specific binding (analyte concentration, mass or other measure of quantity) is determined as the difference from the reference frequency and the resonant frequency of the bound sensor.

The determination of bioanalyte or chemical interaction of the test sensor in the gaseous state is achieved in a similar manner, namely as the difference of the resonant frequency of the reference sensor minus the resonant frequency of the bound (test sensor).

In one embodiment of the sensor 10, monomeric film coatings are deposited onto the gold surface 44 through the combining of their bifunctional reactive groups wherein one end of the molecule serves as an anchoring group to the elemental gold. The surface anchoring group may be selected from a large group of active chemical moeties including thiols, disulfides, trichlorosilanes, trialkoxysilanes, trialcohol and amines. In the process of deposition these chemically reactive groups will be adsorbed to the elemental gold surface in an irreversible manner. Central to the

creation of the molecular recognition surface is the inclusion of a specific (receptor 46) which serves as the specific binding molecule for the analyte of interest. These receptors 46 can include a large series of organic molecules such as biotin, avidin, proteins, antibodies, carbohydrates, nucleic acids, natural or synthetic drugs, other types of antigens, chelating compounds or combinations thereof. These receptors 46 have a known or unknown affinity for the analyte of interest.

In the construction of the biomolecular recognition surface 42, the monomeric films which are anchored to the elemental gold surface 44 through one of their active functional groups also serve to bind the receptor molecules 46 through the second active chemical group. The anchoring of the receptor molecules 46 through the second active group can be achieved through a number of common chemical processes including reactions with amines, carboxylic acids, thiols, succinicanhydrides, alcohols, maleic anhydrides or combinations thereof. Additionally, these active groups which bind and anchor the receptor molecules 46 can be reacted to the monomeric films before or after anchoring of these films to the elemental gold surface 44. Additionally, chemical conversion as well as blocking reactions can be accomplished through processes that occur prior to or after anchoring of these monomeric films to the microbeam structure 38.

A further embodiment of the molecular recognition surface 42 involves polymeric film coatings. The use of polymeric film coatings provide an alternative mechanism of force transduction achieved through mass loading to the underlying beam structure. The creation of force in this case, is mitigated by the change in conformational structure of these polymeric films which are chemically adsorbed to the elemental gold 44 in an irreversible manner. The change in conformational structure of these polymers is associated with a quantifiable force and or stress which is efficiently transduced to the underlying beam structure 38. Therein, the transduction of these forces to the beam structure 38 results in the mechanical deformation of the beam 38, alteration of the crystalline lattice

of the ferroelectric PZT material 22, and hence the creation of electric charge.

Similar to the monomeric films, polymeric films are also bifunctional wherein one region of these polymers comprises a surface adhesive functionality and the other portion comprises a molecular recognition for the receptor molecule. The surface adhesive characteristic of these various polymers is mitigated through reactive chemical groups including thiols, disulfides, trichlorocylines, trialcheoloxilines, triolsilanes, and amines. Similar to the use of monomeric films, the molecular recognition group, or receptor may be comprised of specific functional molecules including peptides, proteins, nucleic acids, drugs, antigens, chelating compounds, carbohydrates, complex sugars, gangliosides, sialic acid or combinations thereof. These receptors have known or unknown affinities for their cognate analytes. The combination of these receptors and polymeric thin films can occur before or after their deposition onto the microbeam structure, and may be mitigated through a serves of reactive groups including amines, carboxylic acids, thiols, succinicanhydrides, maleic anhydrides, alcohols or combinations thereof. Chemical conversion of these reactive groups, similar to the case of monomeric thin films, may occur before or after deposition onto the microbeam structure and involves such reactions as gluteraldehyde coupling, disulfide formation, N-hydroxysuccinimide coupling, amide bond formation, protein A binding or other methods.

Examples of these polymeric film coatings include compounds such as polyethylenamine, amino-polyethylene-glycol (amino-PEG), nucleophylic PEGs such as amino acid esters of PEG, thiol or disulfide PEG, PEG-succinate, carboxyl PEGs, NHSactive esters of PEG or PEG-glycidyl ether (epoxide).

In addition to the bimolecular recognition surface 42, added features of this microelectromechanical sensor 10 include the integration of on-chip circuitry which serves to amplify the outgoing charge produced by the deformation of the ferroelectric PZT films 22.

We claim:

Claims

1. A cantilever microbeam which responds to a chemical stimulus, binding event or mass loading with an electrical output.

2. A device according to claim 1 formed using a series of microfabrication processes, in the micrometer to millimeter size range and using thin deposition of piezoelectric materials.

3. A cantilever microbeam, the microbeam responding electrically to a specific chemical, biochemical, or physical stimulus associated with immobilization of a chemical, biochemical, or cell derived epitope to a cognate molecular recognition surface covering a specific region of the microbeam.

4. A device according to claim 3 wherein the response is a change in resonant frequency.

5. A device according to claim 3 wherein the response is a change in amplitude of vibration to a preset electrical or mechanical input stimulus.

6. A device according to claim 3 wherein the response is a production of a spontaneous charge due to an induced stress.

7. A device according to claim 3 wherein the response is due to a transduction of a mechanical force through a change in the conformation of an organic thin film containing a specific biomolecular receptor, leading to a production of a spontaneous charge

due to an induced stress in the piezoelectric material.

8. A device according to claim 3 further including a monomeric film coating on the microbeam, the coating being composed of bifunctional molecules where one end of the molecule comprises a surface anchoring group and the other end comprises a molecular recognition group, the surface anchoring group being selected from a group consisting of thiols, disulfides, trichlorosilanes, trialkoxysilanes, trialcohol silanes, amines.

9. A device according to claim 8 wherein the molecular recognition group is formed of specific functional ligands having a binding afinity to an analyte to be detected, one or more ligands being selected from a group consisting of biotin, avidin, proteins, antibodies, carbohydrates, nucleic acids, drugs, antigens, chelating compounds, short peptides, trisaccharides, tetrasaccharides, gangliosides, sialic acid or other combinations thereof.

Fig. 1a

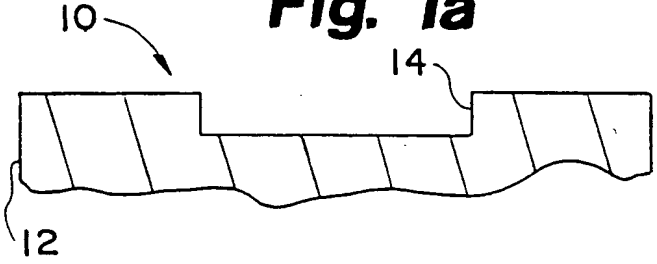


Fig. 1b

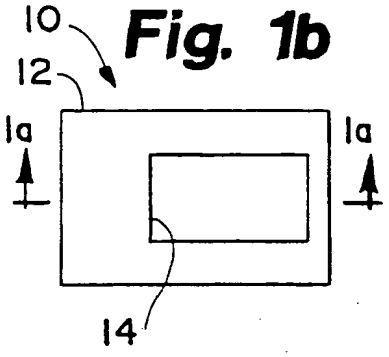


Fig. 2a

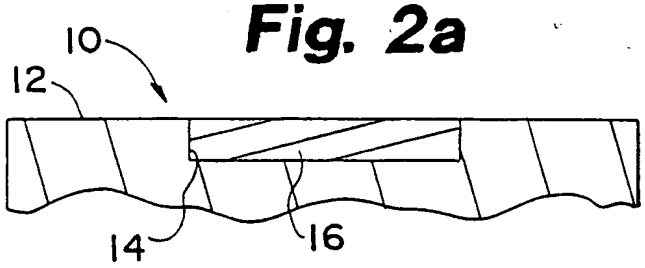


Fig. 2b

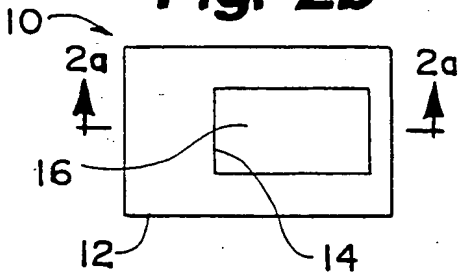


Fig. 3a

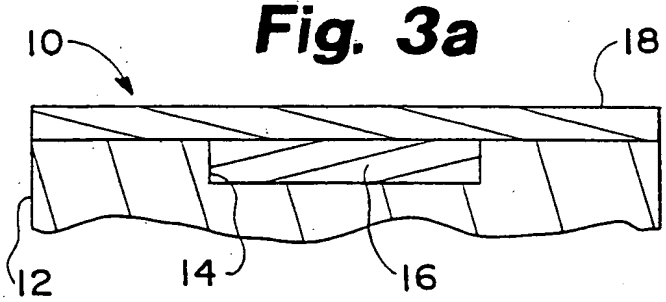


Fig. 3b

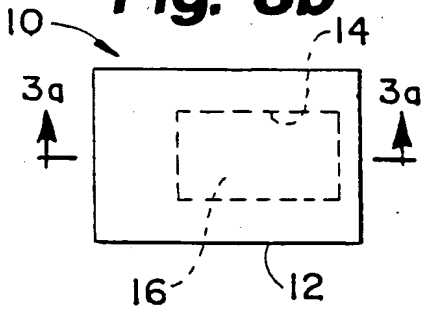


Fig. 4a

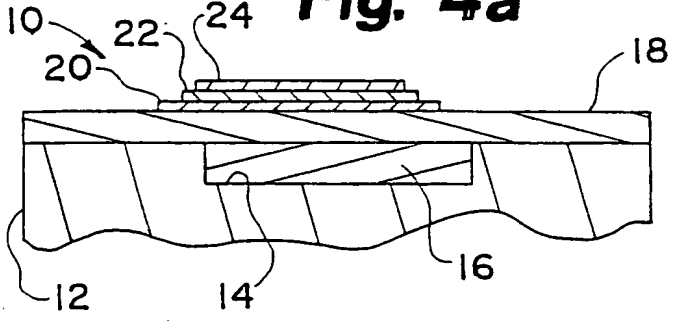


Fig. 4b

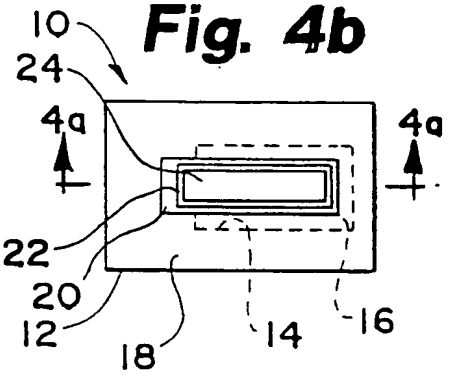


Fig. 5a

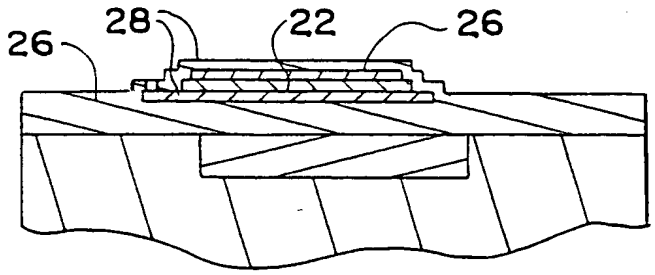


Fig. 5b

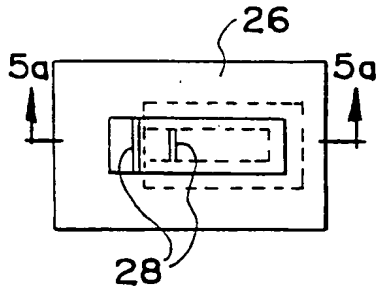


Fig. 6a

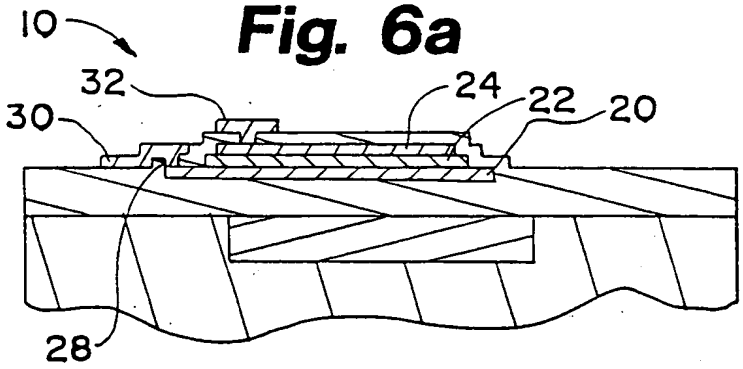


Fig. 6b

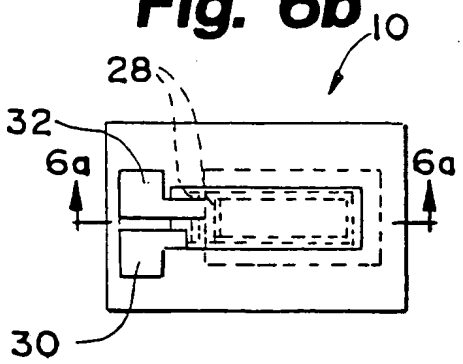


Fig. 7a

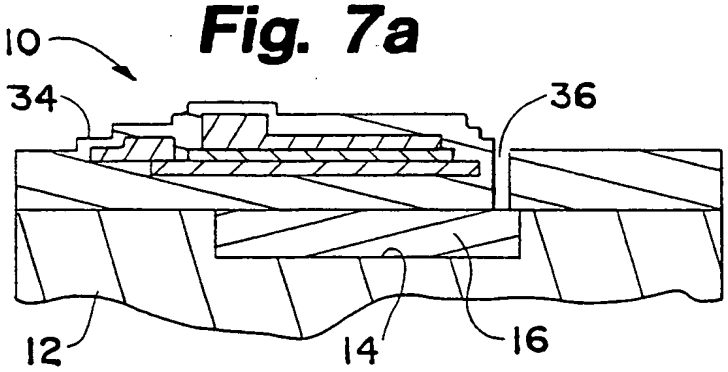


Fig. 7b

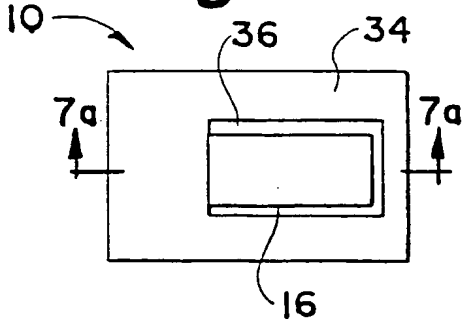


Fig. 8a

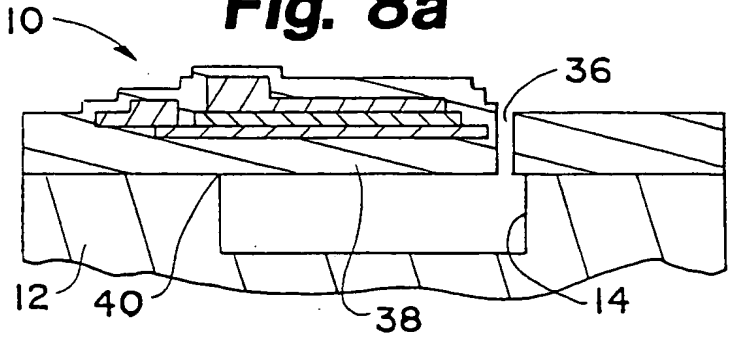


Fig. 8b

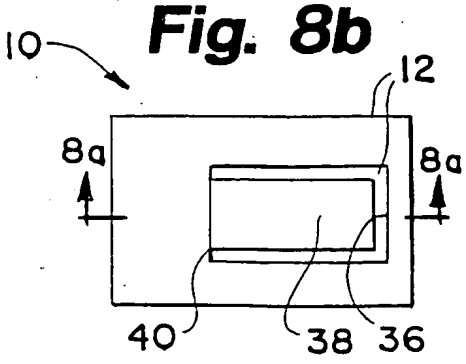


Fig. 9a

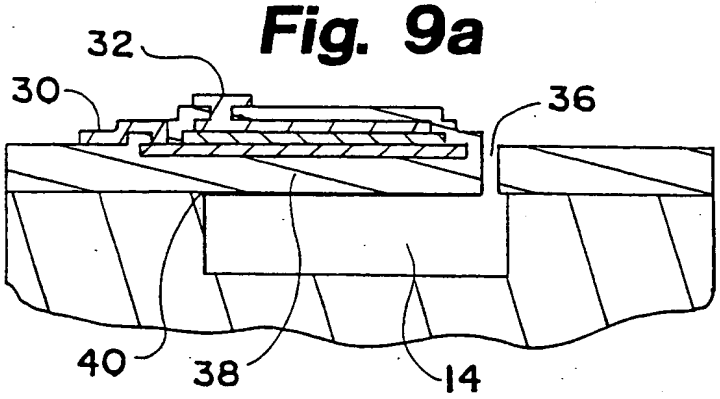


Fig. 9b

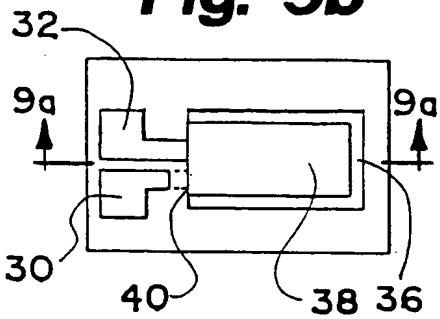


Fig. 10

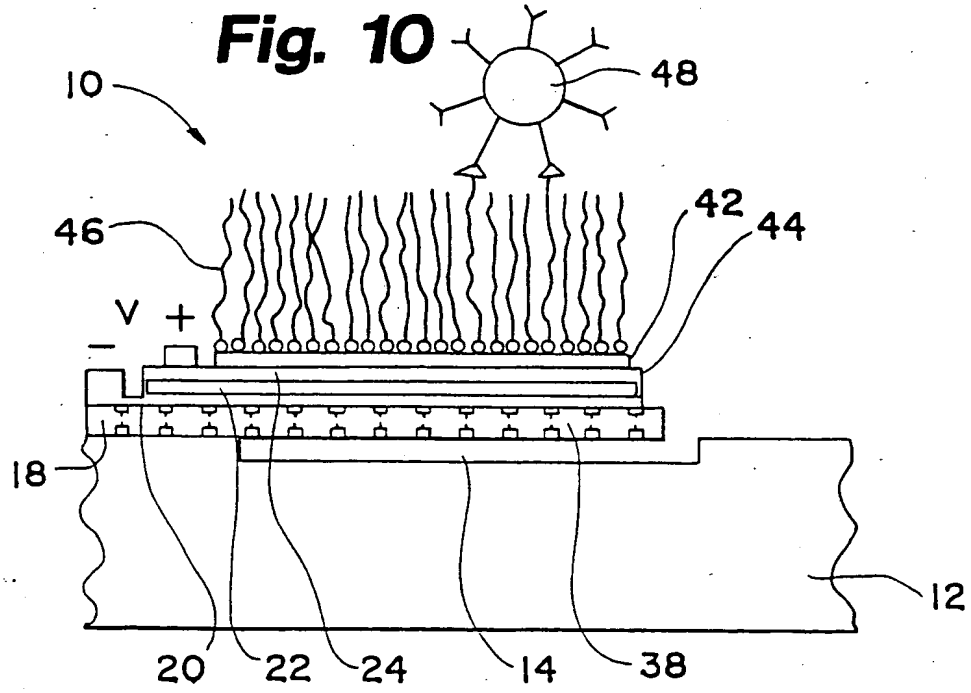


Fig. 11a

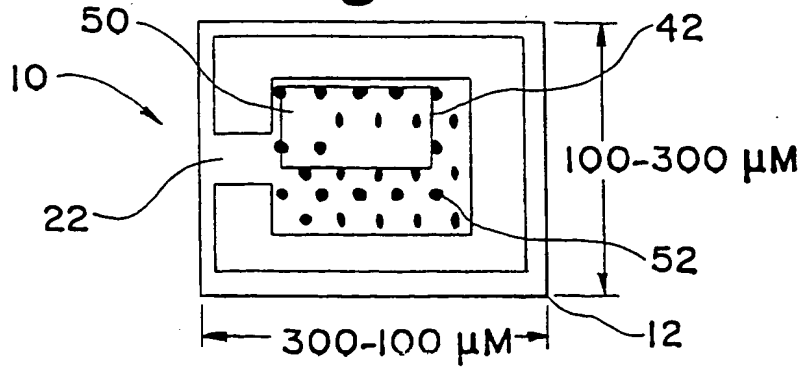


Fig. 11b

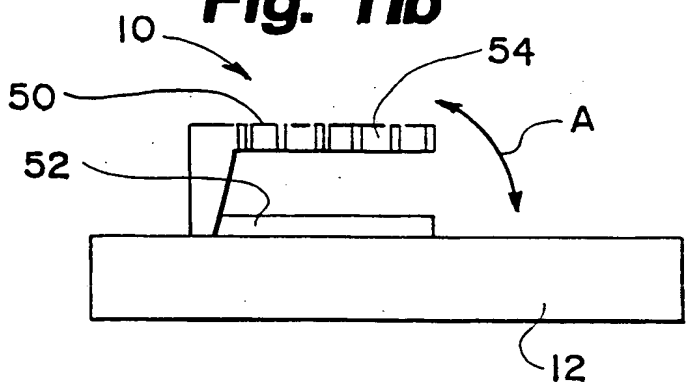


Fig. 12a

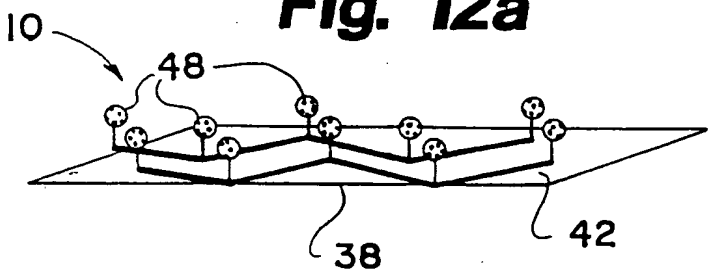
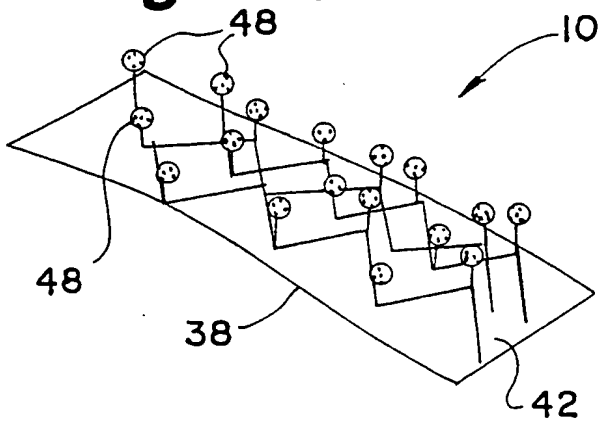


Fig. 12b



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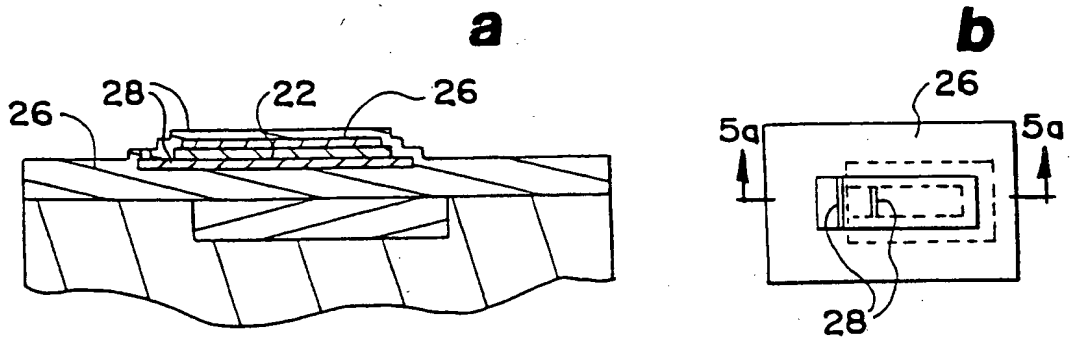
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(57) Abstract

A biosensor includes a cantilever microbeam which responds to a chemical stimulus, binding event or mass loading with an electrical output. The microbeam is formed using a series of microfabrication processes, in the micrometer to millimeter size range and using thin deposition of piezoelectric materials.

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Y	US 4 906 440 A (KOLESAR JR EDWARD S) 6 March 1990 see the whole document see column 3, line 24 - column 3, line 27; claims 1-4	1-4,6
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A	MIHAILOVIC R.E. ET AL: "Measurement of the elastic stress of thin films deposited on gallium arsenide" J.VAC.SCI.TECHNOL.A., vol. 14, no. 4, 1996, pages 2483-2487, XP002091883 US see the whole document	
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